Response to

Department of Health and Human Services
National Institute of Health

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEAM)

Nomination to hold Workshop on Alternative Methods to Replace the Mouse LD50 Assay for Botulinum Toxin Potency Testing

Experts:

Dr Dorothea (Thea) Sesardic
Principal Scientist
Division of Bacteriology
National Institute for Biological Standards and Control (NIBSC)
Hertfordshire, UK

Position: Group leader, bacterial toxin laboratory with >15 years experience on testing of therapeutic formulations of Botulinum toxins.

Dr Rose Gaines Das
Head of Biostatistics
NIBSC

Position: Head of Biostatistics with >25 years experience on validation of assay methods

Dr Russell G A Jones
Senior Scientist
Division of Bacteriology
NIBSC

Position: Expert on botulinum toxins and botulinum anti-toxins with 5 years experience.

List of publication on assay methods for reduction, refinement and replacement for potency assays of Botulinum toxins in support of nomination for NICEATM

In vivo LD50 (reduction/standardisation)

McLellan K, Gaines Das R, Ekong TAN and Sesardic D. (1996). Therapeutic botulinum type A toxin: factors affecting potency. *Toxicon*, **34,** 975-985.

Sesardic D, Gaines Das RE and Corbel MJ (1994). Botulinum toxin: How to define biological activity. *Journal of the Royal Society of Medicine (letter)* **87,** 307.

Sesardic D, Leung T, Gaines Das R (2003). Role of standards in assays of botulinum toxins: international collaborative study of three preparations of botulinum type A toxin. *Biologicals,* 31: 265-276.

Jones RGS, Corbel MJ and Sesardic D. (2006). A review of WHO International Standards for Botulinum Antitoxins. *Biologicals,* (in press).

In vivo non-LD50 (refinement)

Sesardic D, McLellan K, Ekong TAN and Gaines Das R. (1996). Refinement and validation of an alternative bioassay for potency testing of therapeutic botulinum type A toxin. *Pharmacology & Toxicology* 78, 283 - 288.

Sesardic D, Jones RGA, Leung T, Alsop T, Tirney R. (2004) Detection of antibodies against botulinum toxins. *Movement Disorders*, 19: 85-91.

In vitro (replacement)

Ekong TAN, McLellan K and Sesardic D. (1995). Immunological detection of *Clostridium botulinum* toxin type A in therapeutic preparations. *Journal of Immunological Methods* **180**, 181-191.

Ekong TAN, Feavers I and Sesardic D. (1997). Recombinant SNAP-25 is an effective substrate for *Clostridium botulinum* type A endopeptidase activity. *Microbiology*, **143,** 3337-3347.

Ekong TAN, Gee C, Blasi J and Sesardic D. (1997). An alternative bioassay for botulinum neurotoxin type A based on its andopeptidase activity. In Animal Alternatives, Welfare and Ethics (Eds: LFM van Zutphen and Balls M). Developments in Animal and veterinary Sciences 27, 1039-1044.

Sesardic D, Corran PH, McLellan K, Feavers I and Ekong TAN (1997*). In vitro* assays for estimating the activity of therapeutic preparations of botulinum toxin. In: Animal Alternatives, Welfare and Ethics (Eds: LFM van Zutphen and Balls M). Developments in Animal and Veterinary Sciences 27, 1033-1038.

Gaines-Das RE, Heath AB, Martin H and Sesardic D. (1999). Validation of in vitro assays for Botulinum Toxin: A case study. In Alternatives to Animals in the

Development and Control of Biological Products for Human and Veterinary Use. (Eds Brown F, Hendriksen C and Sesardic D). *Developments in Biological Standardization* Vol 101. pp267-276.

Sesardic D, Corran P, Gee C and Ekong TAN. (2000). *In vitro* approaches for estimating activity of tetanus toxin as an alternative assay for specific toxicity. In: "*Progress in the reduction refinement and replacement of animal experimentation*" (Balls M, van Zeller A-M and Halder M Eds.,) pp 969-974.

Sesardic D, Martin H, Tierney R and Bigalke H. (2000). An *in vitro* assay for testing of neutralising antibodies to botulinum toxins. In*: "Progress in the reduction refinement and replacement of animal experimentation*" (Balls M, van Zeller A-M and Halder M Eds.,) pp 1001-1008.

General : reviews and related relevant publications

Corran PH and Sesardic D. (1994). Meeting Report: International Workshop On Bacterial Toxins in Medical Use. *Biologicals* **22**, 83-84.

Sesardic D. (1996). Requirements for valid alternative assays for testing of biological therapeutic agents. *Developments in Biological Standardization* **86,** 311-318.

Brown F, Hendriksen CFM and Sesardic D (Eds). (1999) Alternatives to Animals in the Development and Control of Biological Products for Human and Veterinary Use. *Developments in Biological Standardization* Vol 101.

Sesardic D. (1999). Alternatives to the use of animals for bacterial toxins and antitoxins. In : Celebration of 50 years of progress in biological standardization and control at WHO. *Developments in Biological Standardization*,100:75-82.

Brown F. Hendriksen CFM, Cussler C and Sesardic D. (Eds). (2002). Advancing Science and Elimination of the Use of Laboratory Animals for Development and Control of Vaccines and Hormones. *Developments in Biological Standardization* Vol 111, Karger Press, Basel. Switzerland.

Leung T, Corran P, Gee C, Ekong TAN and Sesardic D. (2002). Application of an in vitro endopeptidase assay for detection of residual toxin activity in tetanus toxoids. In Brown F, Hendriksen CFM, Sesardic D & Cussler K (Eds). Advancing Science and Elimination of the Use of Laboratory Animals for Development and Control of Vaccines and Hormones. *Developments in Biological Standardisation,* 111: 335-340.

Meunier FA, Lisk G, Sesardic D and Dolly OJ. (2003). Dynamics of motor nerve terminal remodeling unveiled using SNARE-cleaving botulinum toxins: the extent and duration are dictated by the sites of SNAP-25 truncation. *Molecular and Cellular Neuroscience*, 22: 454-466.

European Pharmacopoeia Monograph

Botulinum ToxinType A for injection. European Pharmacopoeia 01/2005:2113.

Standard operating procedures

1. Non-lethal mouse local muscular paralysis assay: In vivo assessment of botulinum type A toxin
2. Phrenic nerve hemidiaphragm assay: *In vitro (ex vivo)* assessment of botulinum neurotoxicity.
3. *In vitro* SNAP-25 endopeptidase immunoassay for potency testing of botulinum toxin A preparations.

Data to be presented at the workshop or included in surface mail:

1. **Mouse LD50:** method as in relevant publications. This test was performed at NIBSC from 1991-1996 after which it was replaced by in vivo mouse paralysis test. It is no longer performed at NIBSC for potency testing of therapeutic products since 1996. LD50 data used in comparisons with in vitro SNAP-25 assay were generated by marketing authorisation holders and therefore confidential. Collaborative study compared LD50 assays in 10 laboratories (published data).

2. **Mouse paralysis**: method as in published literature and in electronic version of SOP. Test performed routinely for type A botulinum toxin at NIBSC from 1996-2000 (>10 assays per year) and thereof only for annual re-calibration of product specific reference standards for use in vitro batch release test. Example data with statistical evaluation provided from one recent assay and described in memo from Rose E Gaines Das to D Sesardic 01 March 2006 to be sent in package by surface mail. In house experience also with type B therapeutic toxin. Examples of calibration of product specific reference – confidential information.

3. **Mouse isolated phrenic nerve hemidiaphragm**: method as in electronic version of SOP. At present under in house validation for confirming potency of bulk active toxin and product specific reference standards. Limited and only unpublished data. Example of dose response curve for type A toxin on poster presented on the 5th International Conference on Basic and Theraeutic Aspects of Botulinum and Tetanus Toxins, Botulinum, Denver Colorado, USA June 2005.

4. **SNAP-25**: method as in relevant literature and electronic version of SOP. In routine use at NIBSC since 1999 for batch release of type A botulinum toxin products. Data compared with LD50 using MAH LD50 data. Examples provided for information taking out actual lot numbers but should not be copied without prior consent.

5. **Rat primary spinal cord cell**: preliminary unpublished data on dose response of inhibition of 3H glycine release following incubation with botulinum tpxin A.